

Effect of thyme and myrtle leaves essential oils on the quality characteristics of cured sausage during storage

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Abstract: The aim of this work was to study the effect of adding thyme and myrtle essential oils (TEO and MEO) at different concentrations (0.05 and 1%) on the microbiological and sensorial characteristics of cured sausage during storage (21 days) at +4°C. The chemical composition of essential oils, obtained by hydrodistillation, was analyzed using GC and GC-MS. Twenty and twenty-three compounds were identified for thyme and myrtle essential oils, respectively. The major constituents were described as carvacrol (81.4%) for TEO and 1,8-cineole (61%), α -pinene (23.7%) for MEO. In its second part, the present study was conducted to evaluate the *in vitro* antioxidant and antimicrobial activity of both studied EOs. For this purpose, the DPPH scavenging test and disc-diffusion method was used. Results show that's both essential oils were able to reduce the stable free radical DPPH with an IC₅₀ of 140 (TEO) and 941 mg/mL (MEO). Significant zone of lysis against all the pathogens studied. On comparing the efficiency of both EOs, *T. capitatus* EO exhibited higher antibacterial activity against the majority of strains and especially against *Klebsiella pneumoniae* (DIZ=24.2 mm). During storage, samples containing essential oils showed microbiological parameters stability better than those for the control. The sensorial evaluation shows that 0.05% of either essential oil added was best appreciated by the panelists.

Keywords: Thyme; Myrtle; essential oil; free radical scavenging activity; antibacterial activity; cured sausage.

Introduction

The use of essential oils (EOs) as functional ingredients in foods, drinks, toiletries and cosmetics is gaining *momentum*, both for the growing interest of consumers in ingredients from natural sources and also because of increasing concern about potentially harmful synthetic additives¹. Within the wide range of the above-mentioned products, a common need is availability of natural extracts with a pleasant taste or smell combined with a preservative action, aimed to avoid lipid deterioration, oxidation and microorganism's spoilage.

Until recently, essential oils have been studied most from the viewpoint of their flavor and fragrance chemistry only for flavoring foods, drinks and other goods. Actually, however, EOs and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use. Many authors, in fact, have reported antibacterial²⁻⁴, antifungal^{5,6}, antioxidant and radical-scavenging^{3,7} properties by essential oils and, in some cases, a direct food-related

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DOI: <http://dx.doi.org/>

application has been tested^{8,9}. Among several EOs that may be useful as food preservatives, Thyme (*Thymus capitatus*) and myrtle (*Myrtus communis*) oils may have greatest potential for use in industrial applications¹⁰⁻¹⁵.

In the present study, we are interest to the conservation of a meat product: "salamis", which are widely consumed foodstuffs. In addition to appreciable sensory aspects, salamis have a relatively low price when compared to traditional meat cuts. Generally, the shelf-life of these products is determined by microbiological growth, surface dryness, changes in color and texture, and the development of undesirable rancid flavors caused by lipid oxidation, lipolysis and other reactions¹⁶. Consequently, several synthetic additives such as sodium erythorbate (NaEry), sodium ascorbate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), propyl galate, nitrite (NO₂) and nitrate (NO₃) have been added to sausages to prevent undesirable reactions, thus enhancing the product's shelf-life¹⁷. The use of these additives is however strictly regulated due to its potential carcinogenicity and toxicity^{18,19}.

The objectives of this study were (i) to determine chemical composition of thyme and myrtle EOs by GC/MS (ii) to investigate its antimicrobial and antioxidant activity, and (iii) to evaluate the effect of the addition of different concentrations (0.05 and 1 %) of TEO and MEO on the microbiological and sensory characteristics of cured sausage "salami" during storage compared with synthetic preservative sample.

Experimental section

Reagents

Di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium (DPPH), 2,6-bis(1,1-dimethylethyl)-4-methylphenol (BHT), were procured from Sigma-Aldrich Chemie. α -Thujene, α -pinene, β -pinene, myrcene, γ -terpinene, terpinolene, 1,8-cineole, linalool, methyleugenol, alkane standard solutions (C8-C24) were from Fluka Chemika.

Plant materials

Leaves from wild plants of thyme (*Thymus capitatus*) and myrtle (*Myrtus communis*) were collected in august and january 2010 from the region of Mornaguia (North East of Tunisia) and Ain Draham (North West of Tunisia), respectively. The harvested plants were identified according to Pottier-Alapetite²⁰. Leaves were air dried at room temperature ($20 \pm 2^\circ\text{C}$) for one week, and subsequently assayed for their essential oil composition.

Isolation of the essential oils (EOs)

Samples of 150 g of each organ were submitted to hydrodistillation using Dean-Stark apparatus, for 90 min until there was no significant increase in the volume of oil collection. Afterward, the essential oil was dried over sodium sulfate anhydrous for 15 min and stored in a sealed vial at 4 °C prior to analysis.

GC and GC-MS analysis

GC analyses were performed using a Hewlett-Packard 6890 series gas chromatograph, equipped with a flame ionization detector. A 30 m HP-5MS (5% phenylmethylsiloxane) capillary column, 0.25mm i.d. and 0.25 μm film thickness was employed. Helium was used as a carrier gas at a flow rate of 0.9 mL.min⁻¹. The temperatures of injector and detector were set at 250°C and 280°C, respectively. Oven temperature program was: 40 °C for 1 min, increased

to 250 °C at 2 °C min⁻¹, held for 5 min. Samples were injected into GC using the split mode with a split ratio of 1/10. The GC-MS analysis was carried out on a HP 6890 instrument coupled to a HP 5973N MS computerized system, and equipped with HP-5MS column with the same characteristics as the one used in GC. The ion source temperature was 230°C. The ionization energy was 70eV with a scan of 1 s and mass range of 40-300 amu. The percentage of the compounds was calculated from the GC peak areas, using the normalization method.

Compounds were identified by comparison of their mass spectra with those in the Wiley 238.L mass spectra library. The obtained compounds were also confirmed by comparing their retention indices determined by co-injection of the sample with a solution containing the homologous series of C₈-C₂₂ n-alkanes with the data published in the literature^{21,22}, and whenever possible by co-injection with an internal standard.

Antioxidant activity by DPPH assay

The anti-radical activity of EOs were evaluated using the test of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH)¹⁰. 2 mL of different concentrations of EOs in ethanol and 2 mL of ethanol for control sample were mixed with 2 mL of freshly prepared DPPH solution in ethanol (2.10⁻⁴M) and allowed to stand for 30 min in the dark at room temperature. The absorbance of the solution was measured at 517 nm against ethanol as the blank. The radical scavenging activity was expressed as IC₅₀ (µg/mL), the concentration providing 50% DPPH inhibition. The ability to scavenge the DPPH radical was calculated using the following formula: %Inhibition = [(AC₍₀₎ - AS_(t))/AC₍₀₎] x 100, where AC₍₀₎ is the absorbance of the control at t = 30 min and AS_(t) is the absorbance of the tested sample at t = 30 min. BHT was used as a positive control. Tests were carried out in triplicate.

Antibacterial activity by disc diffusion method

The *in vitro* antibacterial activity of the tested EOs was carried out by disc diffusion method against two-gram positives bacteria (*Staphylococcus aureus* ATCC 25923 and *Streptococcus A* ATCC 11 700) and four-gram negatives (*Pseudomonas aeruginosa* ATCC 9027, *Salmonella enteritidis* ATCC 14 028, *Esherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 13 833). In this test, nutrient agar (NA) was used as culture media²³.

100 µL of suspension of tested microorganisms, containing 10⁷ colony-forming units (CFU)/mL of bacteria cells spread on NA. The filter discs (6 mm in diameter) were individually soaked with 15 µL of essential oils and placed on agar plates which had previously been inoculated with the tested microorganisms. Disc without samples were used as a negative control. Amoxicillin (30 µg/mL) was used as positive reference to compare sensitivity of strain/isolate in analyzed microbial species. The petri dishes were kept at 4 °C for 2 h. The plates were incubated at 37 °C for 24 h.

Antimicrobial activity was evaluated by measuring the diameter of the growth inhibition zones in millimeters (including disc diameter of 6 mm) for the test organisms and comparing to the controls. The measurements of inhibition zones were carried for three sample replications, and values are the average of three replicates.

Salami manufacturing procedure

Salamis were manufactured according to an industrial formulation (Ellouhoum Society) as follows: Sausage batter was prepared by grinding frozen boneless beef meat (75 %) (w/w) and beef fat (25 %) (w/w) to 5 mm size and mincing it to the final size in a bowl-cutter (Rowenta, Universo, Germany). In the original formulation the following ingredients and additives were

added: sodium chloride (28 g/Kg), commercial spice mix (7 g/Kg), glucose (4 g/Kg) and sodium nitrite (150 mg/Kg). This batch corresponds to the positive control batch.

To assess the influence of essential oil addition, sodium nitrite was replaced by different concentrations (0.05 and 1 %) of thyme and myrtle essential oil.

The mixture was stuffed into artificial casing 100×150 mm long, clipped at both ends and cooked in a water bath. The sausages were kept in the bath until 80°C was reached at the coldest point (geometric centre of the cured sausage, which corresponds to the thickest part of the product). When the endpoint temperature was achieved, the sausages were immediately chilled in ice for 30 min and then placed in plastic containers and stored at 4°C for 21 days. Samples were withdrawn from each formula for analysis at 0 time and weekly.

Salami analyses

Microbiological analyses

For microbiological analysis, 10 g of each sample of sausage batch were collected aseptically, transferred into a sterile plastic bag and were homogenized with 90 mL of peptone water. Serial 10-fold dilutions were prepared in sterile peptone water. Appropriate dilution samples (1 or 0.1 mL) were poured or spread in duplicate on different growth media. Total mesophilic flora was enumerated on Plat Count Agar (PCA) after 48 h of incubation at 30 °C; *Enterobacteriaceae* on Violet Red Bile Glucose Agar (VRBG) after 24 h at 37°C; yeasts and molds on Sabouraud Agar after 5 days of incubation at 25 °C and Psychrotroph counts on PCA of incubation at 7 °C for 8 days²⁴.

Sensory evaluation

Samples were presented to trained panelists (n=17) in balanced, random, monadic order in individual booths in a sensory laboratory. In the numerological scale, 1= dislike extremely and 5= like extremely was used to evaluate color and overall acceptance (in terms of odor, flavor and preference) of the samples.

Statistical analysis

Data were presented as the mean of triplicate ± standard deviation (mean ± SD). The data were analyzed for statistical significance using Statgraphics Centurion XVI. Differences between treatments were assessed using one-way ANOVA, followed by Tukey HSD post hoc test. P values below 0.05 were considered significant.

Results and discussion

Chemical composition of EOs

The EOs were analyzed by GC and GC/MS. Twenty and twenty-three components were identified and quantified in thyme and myrtle leaves, which constituted 98.9 and 97.8 % of the total oil, respectively (Table 1). The analyzed oils were dominated by the monoterpene fraction representing 96.8 and 97.9 % in TEO and MEO, respectively, the oxygen-containing monoterpene being the most representative group (72.0–84.8 %) of this fraction and in both oils (Table 1). Furthermore, this analysis revealed that carvacrol was the dominant constituent in TEO with 81.4 % of the total composition. While 1,8-cineole (61%) and α -pinene (23.7%) were the major compounds found in MEO. These results confirm with other authors works that found carvacrol, α -pinene and 1,8-cineole being the major constituents of Tunisian thyme²⁵⁻²⁷ and myrtle oils^{10,28,29}.

Table 1: Essential oil composition (%) of *Thymus capitatus* and *Myrtus communis* leaves.

Components ^a	IR ^b	Percentage ^c (%)	
		MEO	TEO
Isobutyl isobutyrate	921	0.6	-
α -Thujene	928	0.2	0.7
α -Pinene	938	23.7	0.3
1-Octen-3-ol	978	-	0.1
β -Pinene	980	0.5	0.1
Myrcene	991	0.2	0.9
α -Phellandrene	1012	-	0.1
α -Terpinene	1024	-	1.0
p-Cymene	1034	-	4.0
β -Phellandrene	1043	-	0.2
Isobutyl 2-methylbutyrate	1010	0.4	-
δ -3-Carene	1012	0.5	-
1,8-Cineole	1033	61.0	-
γ -Terpinene	1063	0.4	5.3
(Z)-sabinene hydrate	1076	-	0.1
Terpinolene	1093	0.2	0.1
Linalool	1101	1.7	-
<i>trans</i> -Pinocarveol	1139	0.3	-
<i>p</i> -Mentha-1,5-dien-8-ol	1142	0.2	-
Berneol	1188	-	0.4
Terpinen-4-ol	1179	0.8	0.7
α -Terpineol	1189	3.3	-
Geraniol	1257	0.6	-
Thymol	1296	-	0.2
Carvacrol	1316	-	81.4
Carvacrol acetate	1381	-	0.8
exo-2-Hydroxycineole acetate	1354	0.2	-
Geranyl acetate	1384	1.9	-
Methyl eugenol	1404	0.3	-
β -Caryophyllene	1419	0.3	2.2
α -Humulene	1454	0.1	-
β -Bisabolene	1555	-	0.2
Geranyl isobutyrate	1516	0.1	-
Caryophyllene Oxide	1584	0.3	0.1
Classes			
Aliphatic compounds		1.1	
Monoterpene hydrocarbons		25.7	24.5
Oxygenated monoterpenes		70.0	68.6
Benzoid compounds		0.3	

Sesquiterpene hydrocarbons	0.7	4.5
Total (%)	97.8	98.6

^a Compounds identified on the basis of comparison with MS database spectra, retention indices and pure reference chemicals, are listed in order of elution from HP-5MS column.

^b Retention indices relative to C8 – C22 *n*-alkanes on HP-5MS column.

^c Percentage (mean of three analyses) based on FID peak area.

Antioxidant activity

The radical scavenging capacity of the tested EOs increased in a concentration dependent manner. The values for 50% scavenging activity (IC₅₀) are presented in Table 2. The IC₅₀ value is negatively related to the antioxidant activity, the lower the IC₅₀ value, the higher the antioxidant activity.

The TEO showed a radical scavenging activity (IC₅₀= 140.0 ± 3.0 µg/mL) higher than that of MEO (IC₅₀= 941.0 ± 2.0 µg/mL). Moreover, results show that both of oils have an antioxidant activity lower than the synthetic antioxidant BHT (IC₅₀= 20.0 ± 1.0 µg/mL). Antioxidant properties of carvacrol, 1,8-cineole and α-pinene were reported previously³⁰ Therefore, activity of the essential oil could be attributed to high contents of these components present in the oil.

Table 2: DPPH scavenging activity of *Thymus capitatus* and *Myrtus communis* essential oils.

	IC ₅₀ (µg/mL)
TEO	140.0 ± 3.0
MEO	941.0 ± 2.0
BHT	20.0 ± 1.0

Antibacterial activity

Many common pathogenic Gram (+) and Gram (-) bacteria associated with food poisoning and spoilage were challenged with thyme and myrtle EOs and results are summarized in Table 3.

According to these results, both EOs exhibited moderate to strong antimicrobial activity against the tested bacteria. However, the MEO failed to show antibacterial activity forward *P. aeruginosa* (DIZ=9.4±0.1) and *S. aureus* (DIZ= 10.2±0.2). Similar results were obtained by Yadegarinia et al³.

E. coli is the most sensitive microorganism with a diameter zone inhibition about 15.3±0.6 mm, tested in the presence of MEO.

As for *T. capitatus* essential oil, the most sensitive bacteria was *K. pneumonia* (DIZ=24.2±0.8 mm) which was closely followed by *P. aeruginosa* (DIZ=24.0±1.1 mm), *Streptococcus A* (DIZ=22.5±0.2 mm), *Staphylococcus aureus* (21.1±0.4 mm) and *S. enteritidis* (20.1±0.7 mm). This antimicrobial spectrum obtained with the TEO, is comparable in most cases, to the results reported by Sokmen et al.³¹ in a characterization of Turkish thyme (*T. spathulifolius*) essential oil and methanol extracts.

This discrepancy, between used EOs, in inhibiting the tested strains can be explained by the fact that the activity depends on the type, essential oil composition and the type of target microorganism³². Many other factors could also be involved such as insolubility in aqueous media³³, or seasonal and intraspecific variation of EO composition³⁴.

Furthermore, antimicrobial activities of the EOs are difficult to correlate to a specific compound due to their complexity and variability. Nevertheless, some researchers reported that there is a relationship between the chemical composition of the most abundant components in the EO and the antimicrobial activity^{35,36}. For example, 1,8-cineole (abundant in *M. communis* EO) is well-known chemicals having antimicrobial potentials³⁷. On the other hand, based on a report, α/β -pinene (monoterpene hydrocarbons abundant in MEO) had slight activity against a panel of microorganism. As a result of these findings, the higher antimicrobial activities of TEO could be attributed to its particular chemotype characterized by its complexity with oxygenated-hydrocarbons as dominant components and the presence of equivalent amounts of monoterpene hydrocarbons and sesquiterpene hydrocarbons

Moreover, many reports mentioned that carvacrol and thymol and their precursors (*p*-cymene and γ -terpinene), are biologically and functionally closely associated³⁸. In that context, compared to MEO, *p*-cymene was more abundant in the TEO (4.0 %). Meanwhile, the latter EO contains a moderately higher level of γ -terpinene (5.3 % and 0.4 %, respectively). These data corroborate the wide range of effectiveness of each EO when individually tested against the studied microorganisms.

Table 3: Diameter of inhibition zone (DIZ, mm) of thyme, myrtle essential oils and the antibiotic (amoxicillin)

Microorganism	TEO	MEO	Antibiotic
<i>Pseudomonas aeruginosa</i>	24.0±1.1	9.4±0.1	8.4±0.5
<i>Salmonella enteritidis</i>	20.1±0.7	14.1±0.7	21.7±0.6
<i>Esherichia coli</i>	17.6±0.3	15.3±0.6	21.4±0.8
<i>Klebsiella pneumoniae</i>	24.2±0.8	13.4±0.4	22.0±0.8
<i>Staphylococcus aureus</i>	21.1±0.4	10.2±0.2	28.2±1.0
<i>Streptococcus A</i>	22.5±0.2	12.4±0.3	26.7±1.1

Effect of essential oil addition on salamis characteristics

Influence of nitrite and essential oil levels on microbiological parameters

Results concerning the viable counts of total aerobic mesophilic flora, *Enterobacteriaceae*, psychrotrophic bacteria and yeasts and molds during storage of sausages produced with or without EOs are reported in Figure 1.

The microbiological analysis revealed significant differences between control and samples containing essential oils and sodium nitrite. The differences in total viable counts, *Enterobacteriaceae*, psychrotrophic bacteria and yeasts and molds at the beginning of the process could be related to the addition of essential oil and the synthetic preservative. This result is in concordance with those of Skandamis and Nychas³⁹, Busatta et al.⁴⁰, Martín-Sánchez et al.⁴¹, Mohamed and Mansour⁴², Jayasena and Jo⁴³ and Ozogul et al.⁴⁴ who showed that the addition of essential oils to the meat and meat products affect the amounts of these microflora.

Figure 1.a shows the total aerobic mesophilic flora of all sausage preparations. The initial total aerobic mesophilic flora count was an average of 5.2 log CFU/g, and it significantly increased with storage time for the control sample to reach the level of 7.7 log CFU/g. The addition of rosemary or thyme EO to fine paste meat products has been effective against aerobic bacteria⁴⁵. A similar study conducted by the same authors revealed that bologna sausage samples with added oregano EO (0.02%) and orange fiber (1%) exhibited significantly lower aerobic bacteria counts than control samples during storage at 4°C for 24

days⁴⁶. In another study, Oussalah et al.⁴⁷ have evaluated the inhibitory effect of 60 different essential oils on a *Pseudomonas putida* strain of meat origin, associated with meat spoilage. Results show that twenty-eight oils appeared effective, and oregano, savory, Chinese cinnamon, thymol thyme and carvacrol thyme essential oils showed the strongest antimicrobial activity.

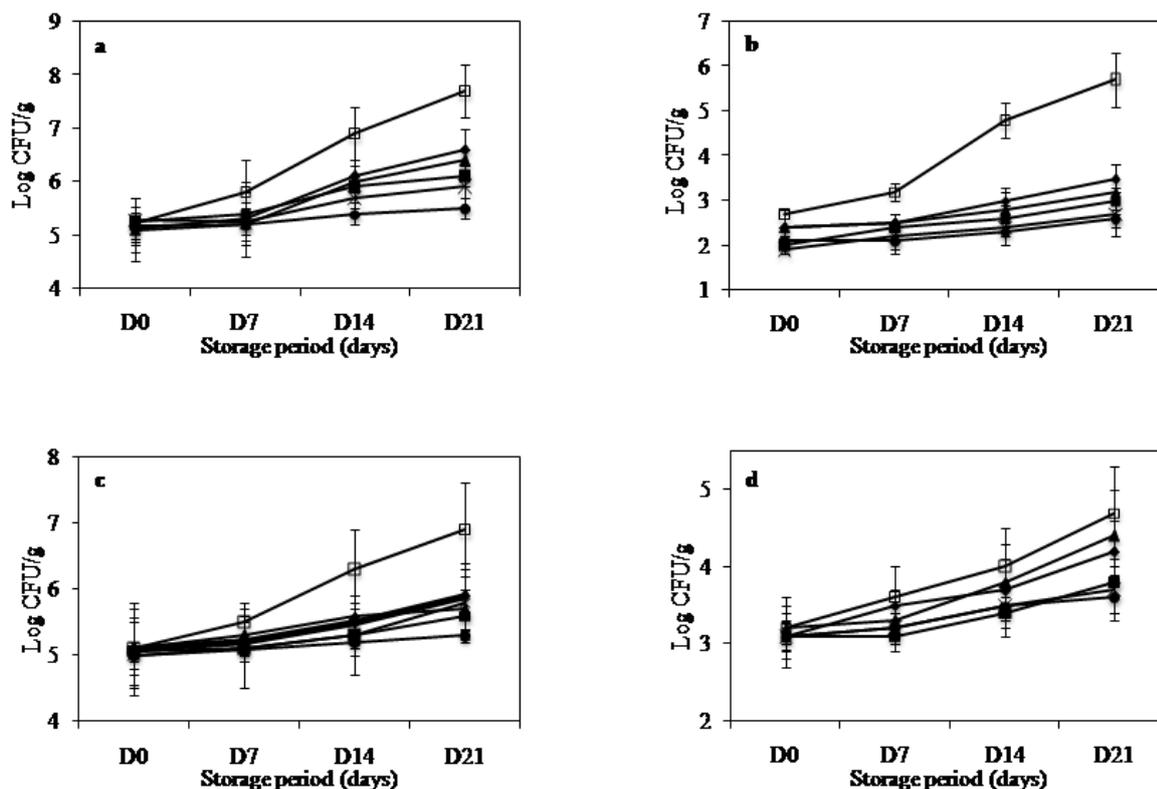


Figure 1: Evolution of Total aerobic mesophilic flora (a) *Enterobacteriaceae* (b) Yeast and mold (c) and Psychrotrophic bacteria (d) during the storage of tested samples: salami with 0.05% MEO (◆); salami with 1% MEO (■); salami with 0.05% TEO (▲); salami with 1% TEO (×); salami with 150 ppm NaNO₂ (●) and control (□)

Initial counts of *Enterobacteriaceae* were about an average of 2.3 log CFU/g for the 6 batches and it depends on the hygienic quality of the raw materials and the handling conditions during processing⁴⁸. They are considered useful indicators of post-processing contamination. This group showed a strong increase (99.88 %) and reached the level of 5.7 log CFU/g for the control sample, whereas, in the sausages added with EOs and NaNO₂, this group showed a slight increase and reached the level of 3.5 log CFU/g for 0.05% MEO, 3.0 log CFU/g for 1% MEO, 3.2 log CFU/g for 0.05% TEO, 2.7 log CFU/g for 1% TEO, and 2.6 log CFU/g for NaNO₂, at the end of storage ($P < 0.05$) (figure 1.b). Comparison with the proposed limits (2–3 log CFU/g) for processed meat⁴⁹ shows that salami added with EOs and preservative were of good quality.

Many studies have reported that plant extracts still used to preserve meat and meat products for their antioxidant and antimicrobial effects^{41,43,50,51}. Antimicrobial effects of basil and thyme essential oil and its major constituents: thymol, *p*-cymene, estragol, linalool, and carvacrol against *Shigella* sp., one of the most important spoilage bacteria in meat, were investigated and found that thyme essential oil, thymol and carvacrol had strong antimicrobial

effects⁵². Penalver et al.⁵³ show that the genera *Thymus* and *Origanum* have an important antimicrobial activity against poultry origin strains of *Escherichia coli*, *Salmonella enteritidis* and *Salmonella essen*, and pig origin strains of enterotoxigenic *E. coli* (ETEC), *Salmonella choleraesuis* and *Salmonella typhimurium*. This finding confirms their potential application in the treatment and prevention of poultry and pig diseases caused by *Salmonella*.

As far as psychrotrophic bacteria, the addition of EOs did not significantly ($P > 0.05$) reduce the psychrotrophic counts in all sausage preparations (figure 1.c). Fat and/or protein may be responsible for the reduction in the antibacterial activity of essential oils in food⁵⁴. In agreement with our results, Mohamed and Mansour⁴² observed a lack of effect of marjoram and rosemary essential oils on the psychrotrophic counts of beef patties during storage. However, in opposition to our results, an *in vitro* inhibitory effect of some essential oils on psychrotrophic growth was observed by Fabio et al.⁵⁵. Further, this study shows that thyme essential oil had the greatest inhibition notably against *Aeromonas hydrophila*.

Similarly finding was observed for the myrtle essential oil⁵⁶, the investigation of the mode of action of the MEO by the time-kill curve against *Listeria monocytogenes* (food isolate) showed a drastic bactericidal effect after 5 min using a concentration of 312 $\mu\text{g/mL}$. These results evidence that the MEO could be used as food antipoisoning agent, particularly, to limit the proliferation of this psychrotrophic pathogen in refrigerated foods.

The number of yeasts and molds increased from about 3.1 log CFU/g to reach about 3.9 log CFU/g for both EOs and nitrite added sausages ($P > 0.05$) (figure 1.d).

These microorganisms have lipolytic activities although there is some controversy about the significance of this activity in the final product. Capita et al.⁵⁷ reported that molds and yeasts are frequently found in low numbers when compared with other microbial groups; consequently, their lipolytic action may be of secondary significance in the manufacture of fermented sausages. But Ferreira et al.⁵⁸ and Ferreira et al.⁵⁹ attributed to this microbial group an important role in the organoleptic profile of the final product.

The treatment with 1% thyme and myrtle essential oil showed the best inhibitory effect against the spoilage bacteria growth at a refrigerated storage. This activity was similarly to that of the synthetic preservative (NaNO_2) with the exception of counts of psychrotrophic bacteria.

A number of reports have indicated that the antimicrobial activity of a given EO can be attributed to its major constituents as well as their interaction with minor constituents present in oils^{2,60}. However, the antimicrobial activity of EOs has been consistently linked to phenolic constituents such as carvacrol, eugenol, and thymol^{61,62}. The presence of hydroxyl groups in phenolic compounds is very vital for their antimicrobial activity. Burt² reported that the antimicrobial activity of EOs is not attributable to one specific mechanism. There are several locations or mechanisms in the microbial cells that supposed to be the sites of action for EO constituents. In brief, EOs can degrade the cell wall, disturb the phospholipid bilayer of the cytoplasmic membrane, and damage the membrane proteins leading to increased permeability of the cell membrane and loss of cellular constituents. They can further disrupt the proton motive force, electron flow and active transport, and coagulate the cell contents². Additionally, these oils can impair a variety of enzyme systems including the enzymes involved in the energy regulation and synthesis of structural components² and inactivate or destroy genetic material⁶³, strengthening their antimicrobial activities.

Sensory analysis

The results obtained in the sensory analysis of sausages added with TEO, MEO and NaNO₂ are presented in figure 2. Both concentrations of the EO had a significant impact ($P < 0.05$) on odor, flavor and preference, evaluated when compared to the salami prepared with the synthetic preservative. Moreover, the two levels of thyme and myrtle EO used differ among them, suggesting 0.05 % the concentration of choice for the same attributes. However, the addition of EO did not influence the acceptance of the color and tenderness for all the samples.

Average flavor evaluations ranged between “neither liked nor disliked” (3) to “liked” (4) for the sausages prepared with 0.05 % EO, while the comments correspond to “neither liked nor disliked” (3) for the sausage prepared with NaNO₂ and ranged from “disliked” (2) to “neither liked nor disliked” (3) for sausages prepared with 1 % EO. The stronger impact of the EO was in the odor and preference, where sausage added with 0.05 % MEO scored between “liked” to “liked extremely”, for both attributes. The results suggest that the addition of 0.05 % EO to salami was acceptable, but the addition of myrtle essential oil was much more appreciated by the consumers. Adjustments in the formulation to reduce the concentration of synthetic preservative may provide a more balanced formulation and result in a more palatable salami while keeping significant antimicrobial activity.

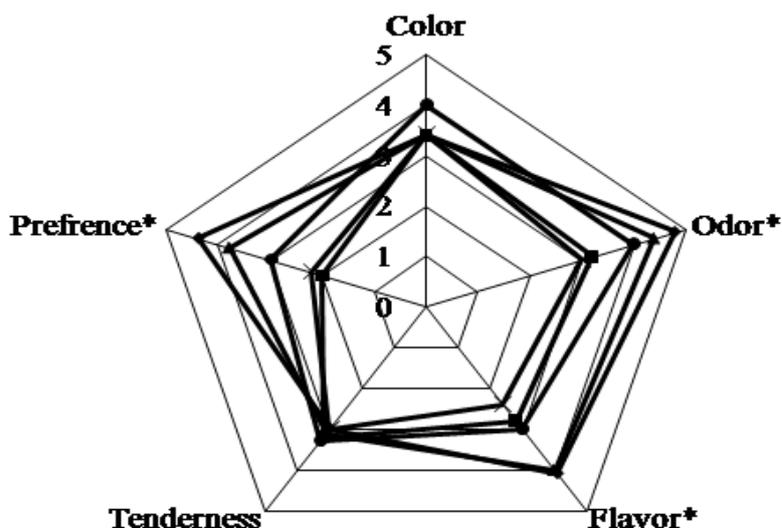


Figure 2: Sensory Evaluation of salamis prepared with: 0.05% MEO (◆); 1% MEO (■); 0.05% TEO (▲); 1% TEO (×) and 150 ppm NaNO₂ (●) (* $P < 0.05$)

Other studies have also demonstrated the sensory viability of adding essential oils to meat products. Marjoram EO added to fresh sausages at 0.11 mL/100 g obtained the same acceptability as the product with no essential oil⁴⁰. The addition of oregano EO (0.01 mL/100 g) to chicken meat promoted desirable odor, according to a panel of trained evaluators⁶⁴. Addition of oregano, rosemary and thyme essential oils at 0.02 mL/100 g in mortadella obtained similar or higher scores than the samples free of essential oil^{45,46}. Furthermore, Martín-Sánchez et al.⁶⁵ have shown that the superficial application of oregano EO to dry-fermented sausages seem to shorten the time necessary for ripening by improving the texture.

Researchers have to be really careful when applying EOs to food products. The strong flavor and aroma present in these oils can be either pleasant or distasteful depending on the type of food in which they are used. Hence, lower levels of EOs can be combined with

existing and novel preservation technologies including low temperature and acidity^{39,66}, modified atmosphere packaging⁶⁷, oxygen absorber⁶⁸, high hydrostatic pressure⁶⁹, preservatives⁷⁰, low-dose irradiation⁷¹ and combination with other antimicrobial compounds⁷².

Conclusion

In the present study, the chemical composition of thyme and myrtle leaves essential oils was characterized by a high number of oxygenated monoterpenes entailing an important antioxidant and antibacterial activities. The overall results of these activities indicate that thyme and myrtle EO can potentially be applied in foods to improve its safety and shelf life. Their application in salamis at the concentration of 0.05% and 1% allowed to improve the hygienic quality of sausages by reducing pathogenic bacteria growth. However, this application is partially limited due to the intense aroma of EOs which may cause negative organoleptic effects. In this study, 0.05% was considered acceptable by consumers for the both EO. Therefore, to ameliorate the microbial stability and the sensory quality of meat and meat products at lower concentration of EO, a combination with other antimicrobial compounds and/or other preservative technologies is important to obtain a synergistic effect without compromising antimicrobial activities.

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